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A STUDY OF THE
CRUDE FIBER IN CERTAIN LEAVES

By

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A Thesis Submitted for the Degree of Master of Arts

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INTRODUCTION

Cellulose has been under investigation for many years. Its chemical composition is known, but the molecular structure is still a mystery. By the term cellulose we understand a variety of substances presenting certain characteristics. These substances are colorless, insoluble in the common, simple solvents, rather resistant to oxidation and hydrolysis; they have the empirical formula $(C_6 H_{10} O_5)_x$ and behave like saturated compounds.

Owing to the difficulty with which these substances can be obtained from the plant in the pure state, the more common method of their determination has been to treat the plant tissue with successive solvents, usually petroleum ether, ether, alcohol, water, dilute alkalies, dilute hydrochloric acid, with occasionally benzol, chloroform and amyl alcohol. The portion which remains insoluble is commonly called crude fiber, supposed to be cellulose, correction being made for the ash content. The above method is used in Dragendorff's Plant Analysis, Helen Abbott Michael's Studies in Plant Chemistry, Experiments performed in the Welcome Research Laboratory, and Parson's Method in Prescott's Organic Analysis.

It is known that the typical celluloses are not separated from plants in the pure state but in chemical union with compounds (pectic bodies) which yield readily to alkaline hydrolysis or to the action of halogens. In this group are included the ligno celluloses of woods, containing keto hexene groups.

Some of the general methods of obtaining cellulose from the raw materials are:--

1. Alkaline hydrolysis i. e., boiling the fiber or tissue

in one to two per cent sodium hydroxide and, after washing, exposing to the action of halogen at ordinary temperature. This treatment is followed by a second alkaline hydrolysis i. e., boiling in alkaline solutions such as sodium sulphite, sodium carbonate, or hydroxide to complete the solution of the hydrolyzed fiber. This solution is then filtered from the non-cellulose product. (Cross and Bevan:--Cellulose--An Outline of the Chemistry of the Structural Elements of Plants, 1903)

2. Another method is to digest the material in the cold for three hours with sulphuric acid (sp. gr. 1.64) containing 72 per cent of acid, using 5 to 8 cc. acid per gram of sample. The material is then heated with 2 to 5 per cent acid under the reflux condenser 6 to 8 hours. Ninety-eight per cent of the cellulose is thus converted into dextrose. Too high temperature should be avoided during the heating since this produces lower organic acids. The material is then neutralized with calcium acetate and the filtrate concentrated to a syrupy consistency. To remove the dextrin, the syrup is treated with absolute alcohol until permanent cloudiness results. The residue is then filtered off, and the filtrate is crystallized. H. Ost and L. Wilkening:--Chem. Ztg. XXXIV, p. 461, (1910).

3. A third method consists of hydrolysis with hydrochloric acid. This method is claimed to have an advantage over the preceding one in which the sulphuric acid forms esters with the polysaccharides which decompose in the presence of dilute acids and yield hydrolyzed products. Fuming hydrochloric acid acts but slowly on cellulose. Hydrochloric acid of 37.6 per cent strength will, with occasional shaking, react in a day. The acid first seemingly breaks up the cellulose

mechanically, then hydrolyzes it with heat, and finally the solution contains glucose. One gram of sample requires 100 cc. acid. Optical rotation is observed after one hour and reaches the value for glucose in twenty-four to forty-eight hours. At first the solution is colorless and clear; after several days, a slight flocculency is apparent, while the solution changes from yellow to dark brown. The syrup is then concentrated under reduced pressure to remove the acid. (Richard Wildstatter and Laszlo Zechmeister. Ber. XLVI, 2401 (1913)

Gilsen obtained cellulose from stems of cabbage by successive treatment with ½ per cent sodium hydroxide, fifteen hours boiling in 2 per cent sulphuric acid, standing fourteen days in twelve parts of nitric acid (sp. gr. 1.15) and eight-tenths parts potassium chlorate, one hour in dilute ammonia at 60°C., washing twice, then drying in alcohol. The fiber he obtained dissolved in Schweitzer's reagent. (Czapek--Biochemie der Pflangen, Vol. I, p. 522)

Cellulose treated with nitric acid (sp. gr. 1.1--1.3) at 80°-100°C. is oxidized to oxycellulose. One part of cellulose is digested at 60°-100°C. with a 40 per cent of an aqueous solution of zinc chloride till it gelatinizes and is then heated on a water bath until it dissolves. The product precipitated by alcohol is a hydrated cellulose. The zinc is removed by digesting with hydrochloric acid and washing. Zinc chloride in twice its weight of hydrochloric acid, 40 per cent strength, dissolves cellulose in the cold. The well-known solution of cellulose in Schweitzer's reagent is not an hydrolysis reaction.

The usual product of complete hydrolysis of cellulose is dextrose though straw cellulose and cereal celluloses have

given mannose besides dextrose, while oxycelluloses obtained by nitric acid treatment yield furfural when hydrolyzed with hydrochloric acid. (Cross and Bevan:--Cellulose)

Schultze and Steiger found that galactose is sometimes a product of cells, while xylose and arabinose have been obtained from seeds. Ber. Chem. Ges. XX. p. 290 (1887). Cross and Bevan in Cellulose mention that investigations of cellulose of green fodder plants long immersed in alcohol produce a greater yield of cellulose by alkaline hydrolysis and oxidation. The following table indicates the results obtained:

Per cent Cellulose Isolated

	Directly	Indirectly	Difference
Leaves	28.2	35.4	7.2
Stems	29.5	34.5	5

Up to this date, we find no literature on ferric chloride hydrolysis.

EXPERIMENTAL

I. Leaves gathered directly from the trees in the late autumn and put in five-pound paper sacks were used in these investigations. After the leaves were air-dried, they were ground in a "universal" vegetable chopper a number of times until well pulverized.

In these hydrolysis experiments some of the leaves were used without preliminary treatment, while in other cases, the leaves were first extracted with various solvents according to a method similar to that of Dragendorff.

A preliminary test was conducted on birch leaves using weighed samples of about two grams. These were treated with approximately ten grams of solid ferric chloride ($Fe_2\ Cl_6.12$

H_2O) in a porcelain evaporating dish over a boiling water bath for four hours. The ferric chloride soon liquified and was absorbed by the leaves yielding a slightly soft, charred-looking mass. After four hours the mass was diluted with about one liter of water well stirred and filtered through cheese-cloth, washing the residue and combining the washing with the filtrate. This filtrate was then treated with freshly precipitated basic lead carbonate made as follows:-- A weighed portion of lead acetate was dissolved in hot water, then an excess of crystallized sodium carbonate dissolved in hot water was added, assuming the reaction to be $2\,Pb\,(C_2H_3O_2)_2 + 2\,Na_2CO_3 + 2H_2O \rightarrow Pb\,(OH)_2.PbCO_3 + 4\,NaC_2H_3O_2 + H_2CO_3$. In practice, when the sodium carbonate was not in excess, the white precipitate remained in suspension a long time. The acetate was removed by washing the mixture twelve to fifteen times with boiling water, the clear supernatent liquid being siphoned off each time. Finally the precipitate was stored in a five-kilogram bottle and carefully corked. The filtrate when treated with basic carbonate effervesced and produced a yellowish brown precipitate of ferric hydroxide. When most of the iron had been removed in this way, a further addition of basic carbonate gave a greenish gray precipitate. The solution was filtered, just made alkaline toward litmus with dilute ammonium hydroxide and then treated with hydrogen sulphide to remove the lead. This treatment had to be repeated three to ten times until all traces of iron and lead were removed. The filtrate was then concentrated on a water bath until a solid began to separate. The concentrated extract was then examined for optical activity.

The extract from 3.6252 g. leaves gave a rotation of

$+0.465^{\circ}$ when examined in a 2dm. tube. Volume of extract was 35 cc. Therefore the sample contained 0.1547 g. glucose calculating by means of the formula $[\alpha]_D = \frac{\alpha \cdot 100}{l \cdot c}$ where $[\alpha]_D =$ $+52.5^{\circ}$ for glucose and assuming the active substance to be glucose. Another portion of the extract treated with Fehling's solution gave good reduction.

II. Small weighed samples of birch leaves were hydrolyzed as above. The solid residue after hydrolysis was collected on a weighed filter-paper, dried in a water-jacket oven and the amount of residue determined.

Observations

Sample Taken	Unhydrolyzed Residue	Per cent of Hydrolyzed and Soluble Matter
3.1730 g.	2.2816 g.	29.1
3.6252 g.	2.4676 g.	31.3
4.0544 g.	3.0034 g.	25.1

III. A one-hundred gram sample of birch leaves was hydrolyzed four hours as previously and the extract, after the iron and lead were removed, was treated with phenylhydrazine and the results were negative. A comparison test with pure glucose was made as follows:--

Two grams of glucose were dissolved in 10 cc. of water and four grams of phenylhydrazine and four grams of glacial acetic acid dissolved in another 10 cc. of water. The mixture was heated in a wide test tube on a water bath for 1½ hours when the osazone slowly separated. The precipitate was filtered and washed with water, then with a little alcohol, and drained well. The precipitate was recrystallized from alcohol. The melting point was found to be 205°C.

The solution obtained from the leaves was treated in a

precisely similar manner, but no osazone was obtained.

IV. Another one-hundred gram sample of birch leaves was extracted successively with boiling water, hot one-per cent sodium hydroxide and hot one-per cent hydrochloric solution for one hour. The residue was well washed, dried, and weighed. The resulting 64.5 grams were hydrolyzed with ferric chloride as before and the purified extract tested for an osazone with negative results.

V. A small sample of Japanese Tree leaves was treated successively with water, one-per cent sodium hydroxide, one-per cent hydrochloric acid, water, and ferric chloride to determine the amounts soluble in each. The results are shown in the following table:

		Per cent
Sample	6.9138 g.	
Water soluble portion	1.5076 g.	21.7
NaOH " "	1.6336 g.	23.6
HCl " "	0.6952 g.	10.5
H_2 O " "	0.0300 g.	0.4
$Fe_2Cl_6.12\ H_2O$ "	0.5926 g.	8.5
Residue	2.4538 g.	35.4
		100.1

VI. A large (one-hundred grams) sample of Japanese Tree leaves was treated with the same solvents as the small smaple in the previous experiment. The residue was eighty grams. After hydrolysis with ferric chloride and subsequent treatment with basic lead carbonate and hydrogen sulphide, the solution was clarified with animal charcoal and evaporated to dryness on the water bath. The residue was treated with absolute alcohol which upon evaporation yielded a trace of white crys-

talline substance. The remainder of the portion insoluble in alcohol was treated with phenylhydrazine as already described in order to see whether it would yield an osazone. The yellow precipitate obtained was washed and recrystallized from absolute alcohol. It consisted of yellowish plates whose melting point was found to be 164°-166°C. They were with difficulty soluble in water at ordinary temperature. They proved to be impure phenylhydrazine acetate.

A comparison test was made using a trace of glucose with an excess of ammonium chloride and subsequent treatment with phenylhydrazine. A slight precipitate was obtained, which under the microscope appeared the same as the crystals last described, namely phenylhydrazine acetate.

The effect of the presence of ammonium chloride on the formation of osazone was still more definitely determined by four tests made as follows:

$\frac{1}{2}$ g. $NH_4Cl + 2$ g. glucose,
1 g. $NH_4Cl + 2$ g. glucose,
2 g. $NH_4Cl + 2$ g. glucose,
4 g. $NH_4Cl + 2$ g. glucose,

Using the method already described.

The presence of ammonium chloride does not interfere with the formation of osazone. This indicates that the former solution containing ammonium chloride and yielding no osazone could not have contained glucose.

VII. A one-hundred gram sample of Birch leaves were extracted successively for seven hours with petroleum ether, (boiling point 48°C) purified ether, absolute alcohol, and water, using an extractor as shown in diagram. The residue was per-

mitted to drain at least two days after each extraction. The residue was finally transferred to a flask adding one liter of one-per cent of sodium hydroxide and heated under a reflux condenser on a water bath. This process was followed by a similar treatment with one-per cent hydrochloric acid. The residue was filtered through cheese cloth, well washed, dried and weighed. Forty-five grams remained. A small portion of this residue was dried in the water-jacket oven to constant weight, then ashed.

Table of observations.

Sample of Residue	Ash	Per cent Ash of the extracted residue
0.5568 g.	0.0264 g.	4.7
0.6774 g.	0.0330 g.	4.8

The major portion (forty grams) was hydrolyzed as usual with ferric chloride. The final extract after clarifying was concentrated. Portions of this were tested as follows:

Test Made	Results Obtained
Fehling's solution........................	Reduction
Molisch's Test...........................	Intense violet blue
HCl sp. gr. 1.06 on boiling............	Negative result with amyl acetate treatment
Saccharimeter............................	1.17° in 2 dm. tube (Soleil Ventzke)
Basic lead acetate.......................	Heavy white precipitate

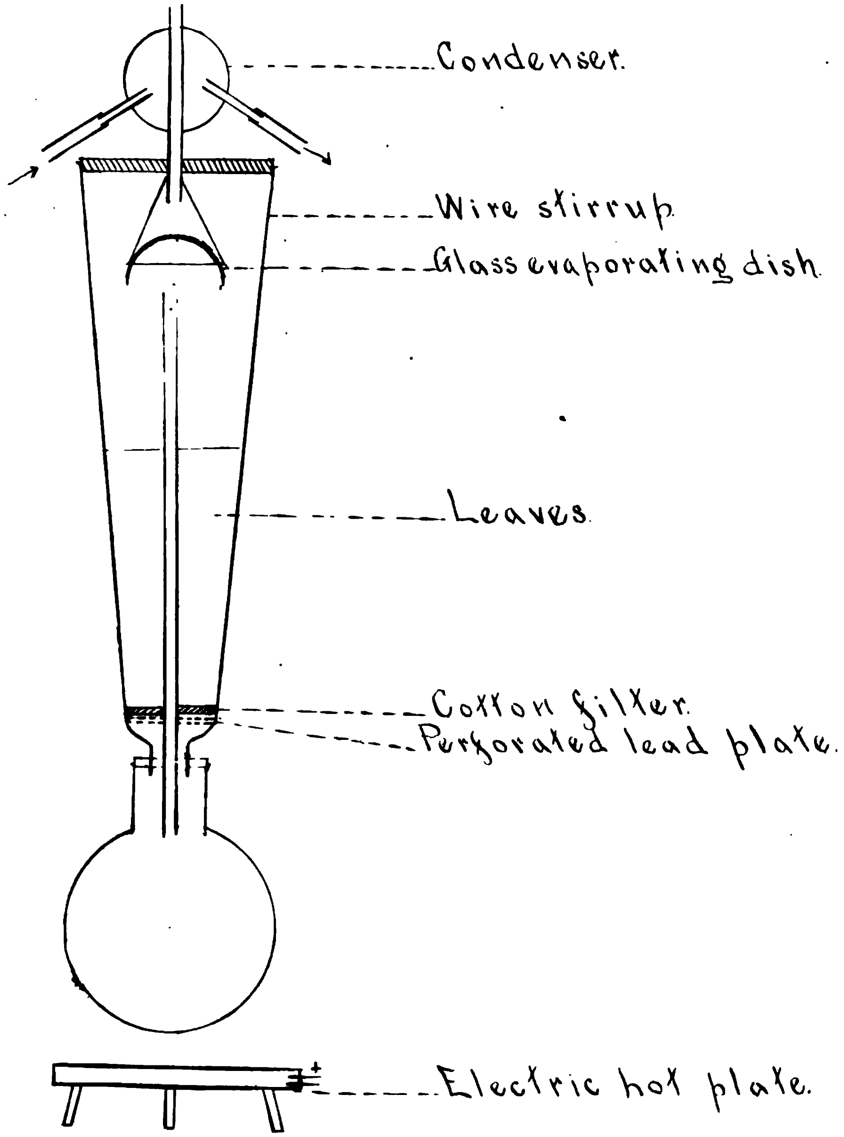
The filtrate after basic acetate treatment did not reduce Fehling's solution hence the reducing substance must have been organic acids.

VIII. A one-hundred gram sample of Maple leaves was extracted successively with petroleum ether, ether, alcohol, water, dilute alkali, and acid as in the last determination.

The residue was dried and a weighed portion treated in the cold with hydrochloric acid (specific gravity 1.2) containing an excess of hydrogen chloride. After ten hours, the material was diluted with one liter of water and filtered. About 100 cc. of this filtrate was treated with basic lead carbonate to remove the hydrochloric acid. The precipitate was filtered off and any lead remaining in solution was removed by hydrogen sulphide. The filtrate here obtained was concentrated. Twenty cc. of this filtrate was examined in the saccharimeter. It was found to be slightly active. The rest of the filtrate from the hydrochloric acid treatment was distilled under reduced pressure (boiling point 48°-55°C). A portion of the dark brown liquid remaining in the flask was treated with phenylhydrazine. No osazone was produced. Another 2 cc. portion of the syrup was diluted to 100 cc., examined in the saccharimeter and found slightly active. These last two tests with the saccharimeter show that the solutions were slightly active, only a few tenths of one degree, very close to the limit of experimental error.

CONCLUSION

The leaves were hydrolyzed with ferric chloride and with hydrochloric acid. The substances produced had reducing properties and showed optical activity, but judging from the fact that no osazones were obtained these products of hydrolysis were not simple sugars.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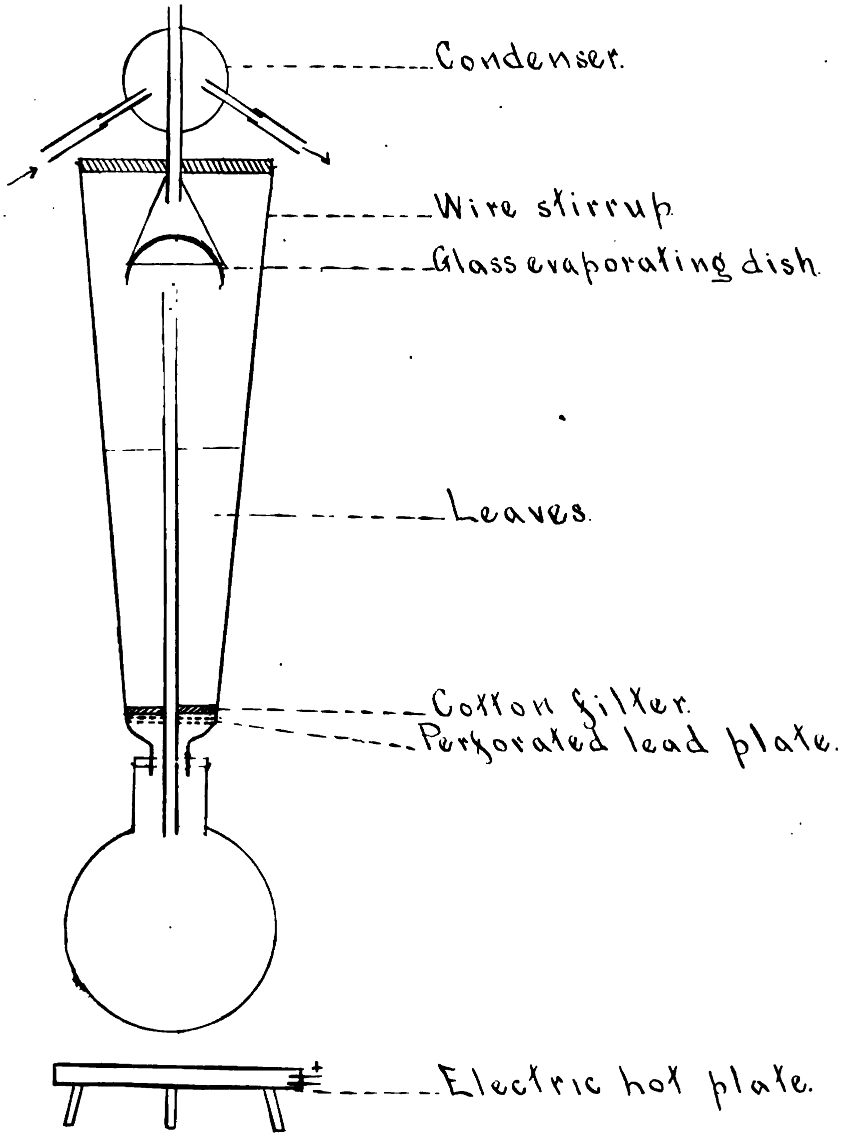
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